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Lungworms (*Metastrongylus* spp.) and intestinal parasitic stages of two separated Swiss wild boar populations north and south of the Alps: Similar parasite spectrum with regional idiosyncrasies

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ABSTRACT

Metastrongylus sp. is a lungworm with worldwide distribution in wild boars and other suids. In Switzerland, two wild boar populations are geographically divided by the Alps. We investigated 84 wild boars, 52 from north and 32 from south of the Alps, different in sex and age (juveniles, subadults, adults), shot between September and December 2018. The lungs were macroscopically examined and dissected for the detection of *Metastrongylus* specimens. Additionally, faecal samples were obtained from 55 animals and analysed by sedimentation/flotation and the mini-FLOTAC® method. Overall, 12′774 *Metastrongylus* sp. specimens were isolated: prevalence was 77.4% and mean burden 196.5 (range: 1–2589), with no significant differences between north (80.8%, 218.0) and south (71.9%, 157.4) nor between sexes and age groups. Macroscopically, dense nodular lesions associated with *Metastrongylus* sp. were present in 19 out of 65 (33.9%) positive lungs. Five *Metastrongylus* sp. were detected: *M. pudendotectus* (67.9%), *M. salmi* (63.1%), *M. confusus* (56%), *M. apri* (44%) and *M. asymmetricus* (17.9%), with a significant difference ($p = 0.012$) between north (32.7%) and south (62.5%) for *M. apri*. The lungworm population was female biased. The number of *Metastrongylus* sp. eggs in faecal samples did not correlate with worm burdens. Furthermore, the following endoparasites were detected: *Isospora suis/Eimeria* sp. (74.5%), *Strongyloides suis* (27.3%%), *Trichuris suis* (20.0%), *Hyostrongylus rubidus/Oesophagostomum* sp. (18.2%), *Globocephalus* sp. (9.1%), *Capillaria* sp. (7.3%), *Ascaris suum* (3.6%), *Giardia* sp. (3.6%) and *Balantidium coli* (1.8%), with significant differences for *S. suis* (north 36.1% > south 10.5%) and *Globocephalus* sp. (only south, 26.3%). Although geographically separated, both Swiss wild boar populations share similar parasite spectra, while also showing some regional idiosyncrasies partially explained by ecological and climatic factors. Despite their clinical relevance being unknown, accurate knowledge concerning the distribution of endoparasites in the wildlife reservoir is relevant to better understand risk factors for the domestic pig population.

1. Introduction

Since the 1980s, the European wild boar population has increased steadily, while the number of hunters has remained more or less the same or has even decreased (Massei et al., 2015). Switzerland is not an exception, as there has been an expansion both in population size and geographical distribution of wild boar since the early 2000s (Meier, 2015). Sharing the same aetiology, wild boars and domestic pigs have a similar aspect and are susceptible to the same pathogens. Wildlife often represents a reservoir of pathogens that can infect domestic animals (Gortázar et al., 2007); therefore, wildlife health represents an important aspect for veterinary medicine. In the case of pigs and wild boars, interactions between the two are rare in general, since pigs are held indoors most of the time. However, with increasing demand for products resulting from organic farming and corresponding better animal welfare closer to natural behaviour, animals are increasingly kept outdoors. Accordingly, the risk of interactions and mutual infections becomes more relevant (Meier and Ryser-Degiorgis, 2018).

Metastrongylus spp. are heteroxenous nematode parasites with suids as definitive hosts. Adult worms live in the bronchi, where the females lay eggs which reach the digestive tract via airways. Earthworms (*Eisenia* spp., *Lumbricus* spp., and others) act as intermediate hosts, which ingest the parasite eggs shed with the faeces of suids. In the earthworm, infectious third stage larvae develop. After the oral intake of

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earthworms by the final hosts, the parasites reach the lungs via mesenteric lymph nodes and the right heart. Finally, adult *Metastrongylus* spp. develop in the bronchi and bronchioles, where they reproduce (Deplazes et al., 2013). *Metastrongylus* sp. infection is reported all around the world in wild boars and pigs (Cleveland et al., 2017; de-la-Muela et al., 2001; Dodangeh et al., 2018a, b; Foata et al., 2006; Järvis et al., 2007; Navarro-Gonzalez et al., 2013; Poglayen et al., 2016; Schubnell et al., 2016; Yoon et al., 2010). Although it is known that wild boars can harbour hundreds of *Metastrongylus* specimens without obvious clinical signs, severe infections can cause verminous pneumonia manifesting as chronic bronchitis and respiratory symptoms, especially in juvenile pigs and wild boars (da Silva and Müller, 2013; Deplazes et al., 2013), suggesting that immunity develops with age (Humbert, 1992). Bronchitis caused by *Metastrongylus* sp. also favours secondary infections with bacteria or viruses, and fatal pneumonia in the course of a lungworm infection is mostly caused by bacterial or viral infections (da Silva and Müller, 2013; Schnieder, 2006).

Today, six *Metastrongylus* species are described worldwide: *Metastrongylus apri* (syn. *M. elongatus*), *Metastrongylus asymmetricus, Metastrongylus confusus, Metastrongylus madagascariensis, Metastrongylus pudendotectus* and *Metastrongylus salmi*. Globally, *M. apri, M. salmi* and *M. pudendotectus* are by far the most commonly reported (Gasso et al., 2014), whereas *M. madagascariensis* has only been reported in Madagascar (Chabaud and Grétillat, 1956). Little is known on the occurrence of helminths in Swiss wild boar, with the exception of *Trichinella* spp., which is considered absent in domestic pigs but possibly present in wild boar (Frey et al., 2009; Gottstein et al., 1997). In Switzerland (size: 41′284 km^2), as with other wildlife species, changes in wild boar population size are estimated based on the hunting bag size, and hunting is regulated by the national hunting law. Supplementary feeding is not allowed and no deworming treatments are performed. Based on hunting statistics, approximately 7′400 wild boars per year are shot. Interestingly, two completely separated populations are present in the country. A large population is present north of the Alps in northern and north-eastern parts of the country, particularly abundant in the areas neighbouring to Germany and France. The other population is spread south of the Alps, neighbouring to the north Italian wild boar population (Meier and Ryser-Degiorgis, 2018b).

The aim of this study was to investigate *Metastrongylus* spp. prevalence and intensity of infection in two separated Swiss wild boar populations north and south of the Alps, to correlate parasite abundance with copromicroscopic findings and to identify gastrointestinal parasites.

2. Materials and methods

2.1. Study area and sampling

Both wild boar populations present in Switzerland (Meier and Ryser-Degiorgis, 2018a) were sampled (Fig. 1). Overall, lungs of 84 wild boars and faecal samples from 55 animals were obtained. From the northern area, the cantonal hunting administrations, hunting parties and gamekeepers of cantons with high wild boar density (Aargau, Zürich, Schaffhausen and Thurgau) were informed and supplied with sampling material and identification forms. Lungs of 52 and faecal samples of 36 animals were collected during a four-month period from September to December 2018. For the southern area, the veterinary office of the canton Ticino provided the samples, which were obtained during a three-week hunting period in September 2018. In this group, a total of 32 lungs and 19 faecal samples were collected. The lungs were frozen at −20 °C and the faecal samples stored at 4 °C until further use.

Sex, weight and age category (juvenile, subadult and adult, determined by dentition (Oroian et al., 2010)) were registered, and the site of death was noted using local designations for meadows and forests. The thawed lungs were photographed and briefly examined for macroscopically visible anomalies, and partial absence of lung tissue due to projectile impact was noted.

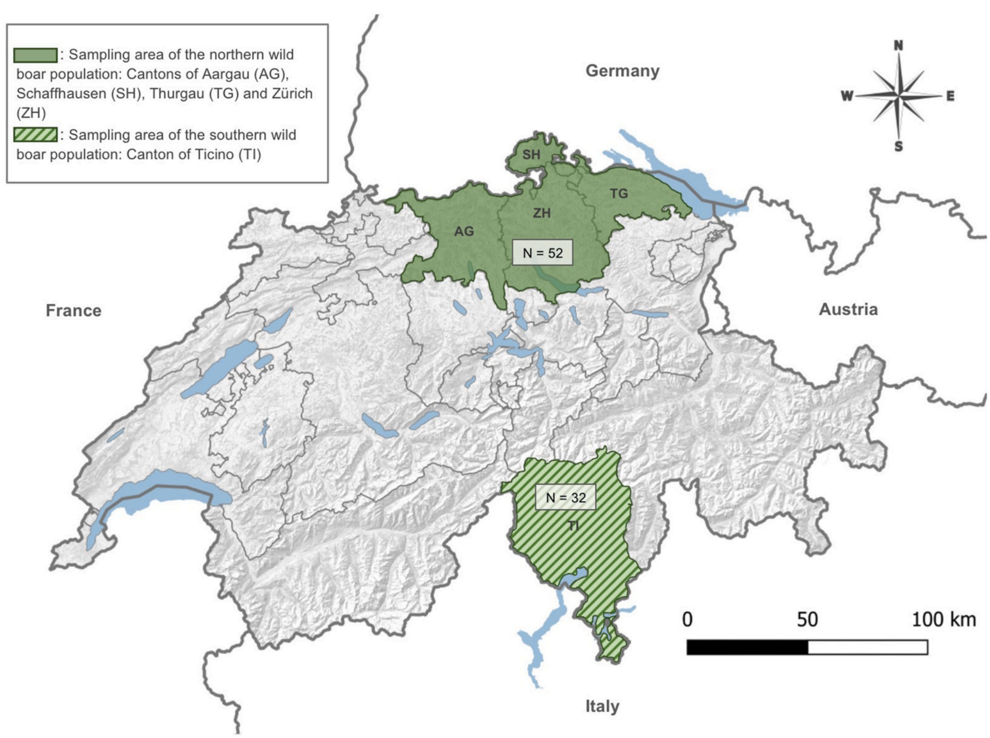

Fig. 1. Map of Switzerland with sampling areas for the northern (Cantons of Aargau (AG), Schaffhausen (SH), Thurgau (TG) and Zürich (ZH)) and the southern (Canton of Ticino (TI)) wild boar population. N: number of lungs sampled.

3. Methods

3.1. Lung necropsy

The lungs were opened along the bronchi and bronchioles and visible worms were collected. The fully opened lungs were then washed in water and the residue was filtered through a sieve (mesh size: 500 µm), from which additional worms were collected. All worms were rinsed off with water and stored in 50 ml tubes containing phosphate buffered saline (PBS).

The nematodes were morphologically classified by sex and species using identification keys (Gasso et al., 2014; Poglayen et al., 2016). Specimens with missing caudal ends were registered as unknown species. For gross examination of the lungs, macroscopically visible pathological changes such as haemorrhages, signs of inflammation, i.e., hyperaemia, excessive mucus, and purulence were noted, as were necrosis and tissue changes such as bronchial wall thickening (Baumgärtner and Gruber, 2020).

3.2. Analysis of faecal samples

The sedimentation/flotation method using zinc chloride (1.45 g/cm^3) solution (Deplazes et al., 2013) was performed to detect parasite eggs and oocysts. In addition, the mini-FLOTAC® method (Cringoli et al., 2017) was used to compare the number of eggs per gram of faecal matter with the number of parasites in the lung. Parasitic stages were identified based on keys from literature (Beugnet et al., 2008; Deplazes et al., 2013). *Metastrongylus* spp. eggs were not differentiated by species, as there are overlaps in size and morphology (Gasso et al., 2014). Also eggs of *Hyostrongylus rubidus*/*Oesophagostomum* and oocysts (*Eimeria* spp. vs. *Isospora suis*) were not further differentiated.

3.3. Analysis of geographic and climatic factors

The geographic differences of the habitats of the two analysed wild boar populations were considered based on the description of the Swiss biogeographic regions (Gonseth et al., 2001). Corresponding climatic factors were analysed using data of the Swiss federal office of meteorology and climatology (Federal Office of Meteorology and Climatology, 2019).

3.4. Data analysis

Prevalence, mean worm burden and range of the worm burden were determined. The Kruskal-Wallis test was used to evaluate differences regarding mean *Metastrongylus* sp. worm burdens between the three age groups or sex of the wild boars. Linear regression analysis was used to evidence potential correlations between parasite load in the lungs and the number of species present (whereby the total number of parasites in the lung was used as the dependent and the number of species as the independent variable) and to detect correlations between parasite load in the lungs and the number of eggs shed with the faeces (with the number of eggs per gram of faeces as the dependent and the total number of parasites in the lung the independent variable). Fisher's exact test was used to compare parasite prevalence between the northern and the southern wild boar populations. All statistics were performed using SPSS® version 25. Values of $p \leq 0.05$ were considered significant.

4. Results

Overall, *Metastrongylus* sp. prevalence was 77.4%, with 65 out of 84 lungs being affected.

4.1. Gross lung examination

By far the most common alteration was laceration combined with massive haemorrhage, lack of tissue and perforation of bone fragments in the lung tissue due to penetration of a bullet (Fig. 2A): 41 out of 84 (48.8%) lungs were heavily damaged, which impeded the detection of other pathological changes. Nineteen of the 65 *Metastrongylus* spp. positive lungs (29.2%) showed dense nodules, especially in the most caudal parts of the lobus caudalis sinister and dexter, which were lighter in colour than the physiological lung tissue (Fig. 2B). Most of these nodular lesions were associated with a conglomerate of nematodes. None of the *Metastrongylus* spp. negative lungs showed these nodular lesions. Three *Metastrongylus* spp. positive lungs showed a yellowish, creamy content within some of the bronchioles, which most likely was purulence, indicating a secondary bacterial infection. In 14 wild boars the lungs showed bronchial wall thickening.

4.2. Prevalence and intensity of infection with Metastrongylus spp. in wild boars

The parts with the most frequent finding of nematodes were the lobi caudales, in which the majority of positive lungs showed a consistent accumulation of *Metastrongylus* spp. in the main bronchii and in the most caudal bronchioli (Fig. 2C). In other lobes, the parasites were more scattered, with occasional nematode aggregations. Prevalence, mean worm burdens and overall ranges and divided by the northern and southern wild boar population are summarized in Table 1. There was no significant difference of prevalence between the two populations nor between female and male wild boar. Also considering the three age categories, prevalence data were overlapping and not significantly differing.

Overall, 12′774 nematodes of the genus *Metastrongylus* were collected. Regarding worm abundance, 31 animals (36.9%) had ≤ 100, 21 (25%) had 101 - 250, 13 (15.5%) had > 250, and 19 (22.6%) had no *Metastrongylus* spp. in their lungs. Overall mean worm burden was 196.5 parasites per lung, with a range from 0 to 2589 specimens. Mean worm burdens and worm ranges again were comparable between the two wild boar populations. Mean worm burden in female and male animals was 177.6 and 221.9, respectively (Table 1). There was no significant difference regarding sex (Kruskal-Wallis test: $H = 0.009$, $p = 0.925$ for the northern population; $H = 0.235$, $p = 0.628$ for the southern population) or the three age groups ($H = 2.73$, $p = 0.255$ for the northern population; $H = 3.34$, $p = 0.188$ for the southern population). However, there was a trend of lower worm burdens in animals older than three years (Tab 1).

Metastrongylus spp. were morphologically assigned to one of five *Metastrongylus* species (Fig. 3); from 1′741 (13.6%) specimens the species could not be determined due to loss of the caudal end (Table 2). The following *Metastrongylus* prevalences in the lungs of the analysed wild boars were obtained: *M. pudendotectus* 67.9% (69.2 vs. 65.6% for the northern and the southern population, respectively), *M. salmi* 63.1% (63.5 vs. 62.5%), *M. confusus* 56.0% (55.8 vs. 56.3%), *M. apri* 44.0% (32.7 vs. 62.5%), *M. asymmetricus* 17.9% (23.1 vs. 9.4%). The difference for *M. apri* was statistically significant ($p = 0.012$).

4.3. Metastrongylus spp. individual counts, sex ratios and number of identified species per wild boar

The species by far highest in number of specimens was *M. pudendotectus,* followed by *M. salmi*, *M. confusus*, *M. apri* and *M. asymmetricus*. This sequence was accordingly observed in the southern population, while *M. asymmetricus* was more frequent than *M. apri* in the northern population (Table 2).

Overall, 9′085 of the 12′774 (71.1%) nematodes were females and 3′689 (28.9%) were males, resulting in a sex ratio of 1 : 2.46 (male vs. female). Sex ratios of the five *Metastrongylus* spp., also separated for the two wild boar populations, are displayed in Table 3: sex ratio was always in favour of females, with the exception of *M. asymmetricus* in the southern wild boar population (1: 0.79).

Concerning the number of nematodes per species in the three age

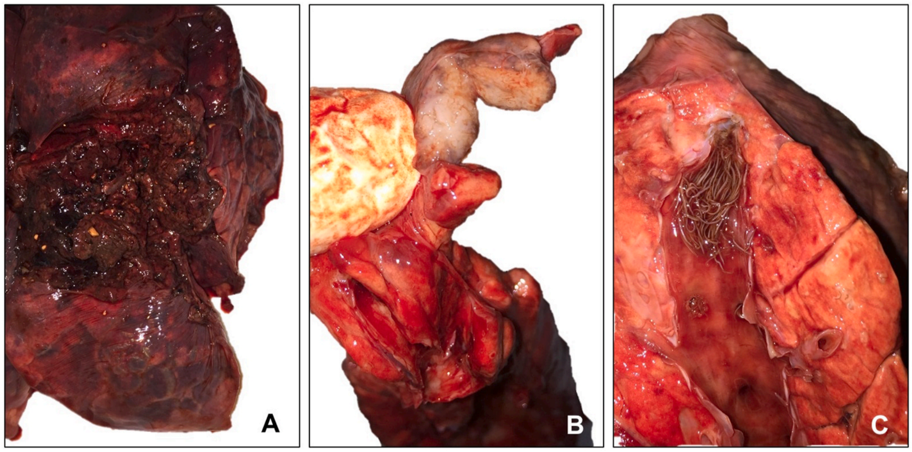

Fig. 2. Wild boar lung with massive trauma after bullet penetration and visible bone fractures (A), dense nodule of the lobus caudalis dexter (B), and accumulation of nematodes in a bronchus (C).

Table 1
Prevalence, mean worm burden and worm ranges of *Metastrongylus* spp. in lungs of 84 wild boars shot north (N, n = 52) and south (S, n = 32) of Switzerland, and divided by sex and age. CI: confidence intervals.

	All animals (n = 84)	*Metastrongylus* sp. positive animals (n = 65)				
		n	%	95% CI	Mean worm burden	Range
N	52	42	80.8	67.5–90.4	218.0	0–2859
S	32	23	71.9	53.3–86.3	157.4	0–597
Total	84	65	77.4	67.0–85.8	196.5	0–2589
Sex		n	%	95% CI	Mean worm burden	Range
Female	50	38	76.0	61.8–86.9	177.6	0–886
N	31	25	80.6	62.5–92.5	183.1	0–886
S	19	13	68.4	43.4–87.4	170	0–551
Male	34	27	79.4	62.1–91.3	221.9	0–2589
N	21	17	81.0	58.1–94.6	269.3	0–2589
S	13	10	76.9	46.2–95.0	141.1	0–597
Age		n	%	95% CI	Mean worm burden	Range
Juvenile (<1 y)	40	30	75.0	58.8–87.3	218.6	0–886
N	28	22	78.6	59.0–91.7	222.4	0–886
S	12	8	66.7	34.9–90.1	208.1	0–551
Subadult (1–3 y)	36	31	86.1	70.5–95.3	187.5	0–2589
N	18	16	88.9	65.3–98.6	241	0–2589
S	18	15	83.3	58.6–96.4	130.4	0–597
Adult (>3 y)	8	4	50	15.7–84.3	101	0–177
N	6	4	66.7	22.3–95.7	100.9	0–177
S	2	0	0.0	0.0–77.6	0	–

groups, this was only significantly different for *M. asymmetricus*: adult wild boars had significantly higher amount of this species in their lungs than subadult or juvenile animals ($F = 5{,}783$, $p = 0.004$).

Out of the 42 positive animals of the northern population, 4 (9.5%) were infected with 5 *Metastrongylus* species, 9 (21.5%) with 4, 16 (38.1%) with 3, 10 (23.8%) with 2 and 3 (7.1%) with one *Metastrongylus* species. The southern population included 23 positive wild boars, of which only 1 (4.3%) harboured all 5 *Metastrongylus* species mentioned above, 14 (61.0%) were infected with 4 species, 5 (21.7%) with 3, 3 (13.0%) with 2, while animals with only one *Metastrongylus* species were not found.

Linear regression analysis identified a significant correlation between the number of nematodes in the lungs and the number of different *Metastrongylus* spp. This was shown for both populations: the number of *Metastrongylus* species increased with the size of the parasite burdens (northern population: $R^2 = 0.114$, $p = 0.008$; southern population: $R^2 = 0.252$, $p = 0.002$).

4.4. Faecal samples

Overall prevalence for *Metastrongylus* spp. eggs was 72.7%, with 72.2% and 73.7% for wild boars belonging to the northern and southern population, respectively (Table 4). The eggs of *Metastrongylus* spp. (Fig. 4) were not differentiated to the species level. *Metastrongylus* nematodes were found in the lungs of all *Metastrongylus* spp. egg excreting animals. Vice versa, not every wild boar with *Metastrongylus* specimens in the lungs was coproscopically positive for *Metastrongylus* eggs in the faeces: two faecal samples from the northern population were negative for any parasitic stages: one was collected from a juvenile male animal, the other from an adult female. Linear regression analysis showed that there was no significant correlation between the number of *Metastrongylus* sp. in the lungs and the *Metastrongylus* sp. eggs shed in the faeces ($F = 1.386$, $p = 0.248$).

Further parasitic stages present in the faecal samples are listed in Table 4. The prevalence of *Isospora suis/Eimeria* sp. was comparably high (74.5%) as *Metastrongylus* sp., while *Strongyloides suis* (syn. *S. ransomi*) (27.3%), *Trichuris suis* (20.0%), *Hyostrongylus rubidus/Oesophagostomum* sp. (18.2%) were less frequent and *Giardia* sp., *Ascaris suum*, *Capillaria* sp., *Globocephalus* sp. and *Balantidium coli* had a prevalence below 10%. *Strongyloides suis* was significantly more frequent in the northern than in the southern population (36.1% vs. 10.5%, $p = 0.004$). In contrast were eggs of *Globocephalus* sp. only detected in wild boars from the south of Switzerland (26.3% vs. 0%, $p = 0.003$). For the other identified parasitic stages (protozoan oocysts/cysts, eggs of *Trichuris suis, Hyostrongylus rubidus/Oesophagostomum* sp., *A. suum, Capillaria* sp.) no significant difference in prevalence was observed between the northern and the southern wild boar population.

5. Discussion

The overall prevalence (77.4%) for *Metastrongylus* spp. infection in Switzerland detected in this study is lower than the one described in adjacent countries. In southern Germany, the prevalence for metastrongylid infections in wild boar was 93.5% (Barutzki et al., 1990), in Italy 96.5% (Poglayen et al., 2016) and in France, a prevalence of 92% was detected (Humbert and Henry, 1989). However, these wild boar populations indeed are present in neighbouring countries but are

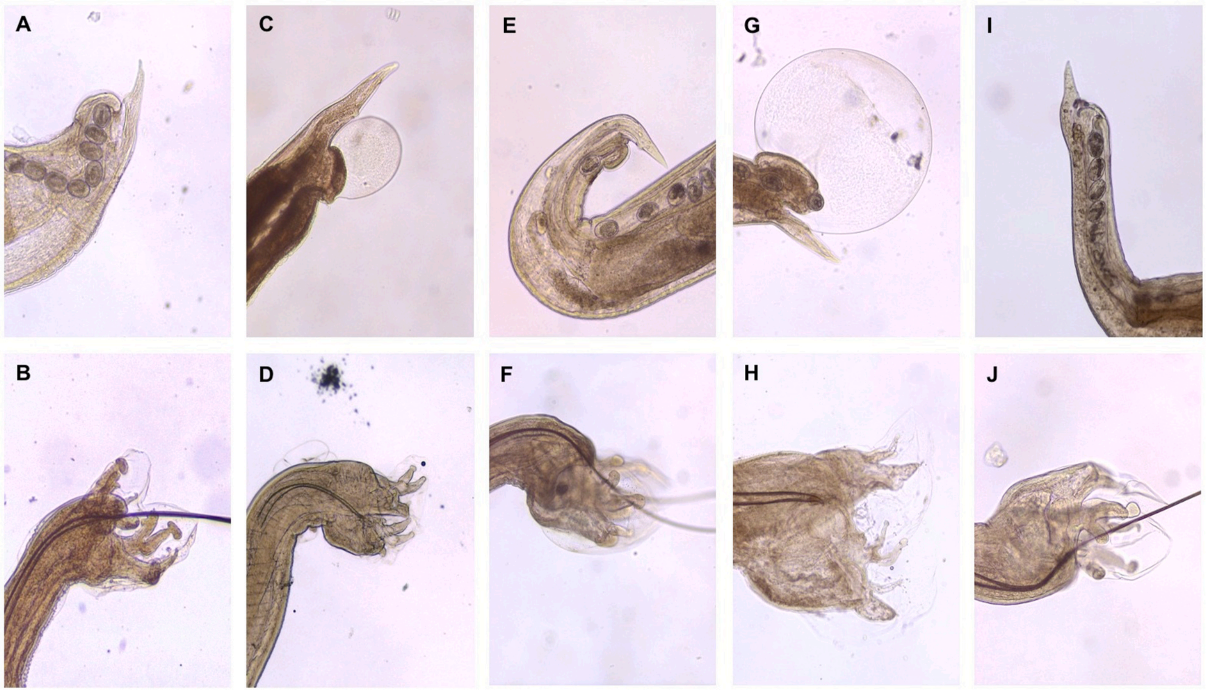

Fig. 3. Caudal ends of the 5 *Metastrongylus* species identified in this study: *M. apri* female (A) and male (B), *M. asymmetricus* female (C) and male (D), *M. confusus* female (E) and male (F), *M. pudendotectus* female (G) and male (H), *M. salmi* female (I) and male (J).

Table 2
Metastrongylus specimens identified in lungs of 84 wild boars shot north (N, n = 52) and south (S, n = 32) of Switzerland. CI: confidence intervals.

Population	*M. pudendotectus* n (%, 95% CI)	*M. salmi* n (%, 95% CI)	*M. confusus* n (%, 95% CI)	*M. apri* n (%, 95% CI)	*M. asymmetricus* n (%, 95% CI)	Species not determined n (%, 95% CI)	Total
North	5′506 (60.1, 59.1–61.2)	1′502 (16.3, 15.7–17.2)	758 (8.3, 7.7–8.9)	125 (1.4, 1.1–1.6)	140 (1.5, 1.3–1.8)	1′123 (12.3, 11.6–13.0)	9′154
South	1′787 (49.4, 47.7–51.0)	717 (19.7, 18.5–21.1)	268 (7.4, 6.6–8.3)	205 (5.7, 4.9–6.5)	25 (0.7, 0.4–1.0)	618 (17.1, 15.9–18.3)	3′620
Total	7′293 (57.1, 56.2–58.0)	2′219 (17.4, 16.7–18.0)	1′026 (8.0, 7.6–8.5)	330 (2.6, 2.3–2.9)	165 (1.3, 1.1–1.5)	1′741 (13.6, 13.0–14.2)	12′774 (100%)

Table 3
Sex ratio (male: female) for *Metastrongylus* species identified in lungs of 84 wild boars shot north (N, n = 52) and south (S, n = 32) of Switzerland.

Population	*M. pudendotectus*	*M. salmi*	*M. confusus*	*M. apri*	*M. asymmetricus*	Overall
North	1 : 2.09	1 : 2.58	1 : 2.87	1 : 1.78	1 : 2.41	1 : 2.41
South	1 : 1.97	1 : 3.24	1 : 2.57	1 : 1.89	1 : 0.79	1 : 2.6
Total	1 : 2.05	1 : 2.77	1 : 2.78	1 : 1.84	1 : 2	1 : 2.23

geographically not in direct contact with the two investigated Swiss populations. Globally speaking, there are also countries/regions with lower prevalences, such as Romania, with 2.1% (*M. apri)* up to 33.21% (*M. salmi)* (Dărăbuş et al., 2019), Bulgaria with 28.75% (Panayotova-Pencheva and Dakova, 2018), Portugal with 42.2% (de Sousa et al., 2004), and similar prevalences to Switzerland, such as Croatia with 66.7% and 80.9% (*M. apri* and *M. pudendotectus,* respectively) (Rajković-Janje et al., 2002), Iran with 68% (Mansouri et al., 2016), Denmark with 79.5% (Petersen et al., 2020) or Estonia with 82% (Jä;rvis et al., 2007).

Explanations for higher prevalences may be linked with the geographical context influencing the wild boar density. For example, the samples investigated by Poglayen et al. (2016) were collected from a game preserve in central Italy, where the animals cannot leave the area due to fences; also, the wild boars from the study of Barutzki et al. (1990) were held in enclosures, leading to higher animal densities than in free ranging boars. With this artificial accumulation of definitive hosts of *Metastrongylus* spp., the infection pressure rises, premising that intermediate hosts (earthworms) are present.

We investigated two geographically separated populations in this study, one roaming north and the other south of the Alps, implying different geographic and climatic conditions (Gonseth et al., 2001). Multiple factors may directly or indirectly influence the infection pressure of nematode infections (Rose et al., 2016). Although it is known that earthworms, intermediate hosts of *Metastrongylus* spp., surface after rainfall (Roots, 1955) and annual precipitation is higher in the southern part of Switzerland (Federal Office of Meteorology and Climatology, 2019), *Metastrongylus* spp. prevalence was by trend lower in the south (71.9 vs. 80.8%). Higher animal density was observed in the north (Federal Statistical Office, 2019), indicating particularly suitable climatic and/or diet-related conditions. Studies showed that the diet of wild boars is extremely diverse (Ballari and Barrios-Garcia, 2013), so there could be several reasons for a varying uptake of earthworms. A source of food which provides similar nutrients to earthworms, but is

Table 4
Parasitic stages in faecal samples from Swiss wild boars (n = 55), separated for northern (n = 36) and southern (n = 19) population; * significant differences between populations, different letters mean significant differences.

Species	Northern population N, % (95% CI)	Southern population N, % (95% CI)	Total N, % (95% CI)
Isospora suis/Eimeria sp.	26, 72.2 (54.8–85.8)	15, 78.9 (54.4–93.9)	41, 74.5 (61.0–85.3)
Metastrongylus spp.	26, 72.2 (54.8–85.8)	14, 73.7 (48.8–90.9)	40, 72.7 (59.0–83.9)
Strongyloides suis *	13, 36.1[a] (20.8–53.8)	2, 10.5[b] (1.3–33.1)	15, 27.3 (16.1–41.0)
Trichuris suis	7, 19.4 (8.2–36.0)	4, 21.1 (6.1–45.6)	11, 20.0 (10.4–33.0)
Hyostrongylus rubidus/ Oesophagostomum sp.	6, 16.7 (6.4–32.8)	4, 21.1 (6.1–45.6)	10, 18.2 (9.1–30.9)
Globocephalus sp.*	0, 0.0[a] (0–8.0)	5, 26.3[b] (9.1–51.2)	5, 9.1 (3.0–20.0)
Capillaria sp.	2, 5.6 (0.7–18.7)	2, 10.5 (1.3–33.1)	4, 7.3 (2.0–17.6)
Ascaris suum	2, 5.6 (0.7–18.7)	0, 0 (0–14.6)	2, 3.6 (0.4–12.5)
Giardia sp.	0, 0.0 (0–8.0)	2, 10.5 (1.3–33.1)	2, 3.6 (0.4–12.5)
Balantidium coli	1, 2.8 (0.1–14.5)	0, 0.0 (0–14.6)	1, 1.8 (0–9.7)

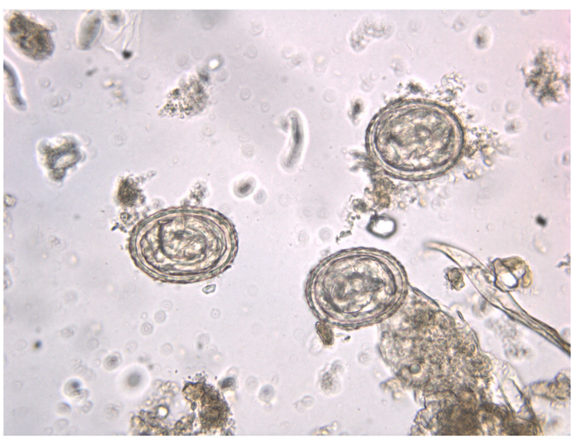

Fig. 4. *Metastrongylus* spp. eggs from a wild boar faecal sample.

easier to obtain or more abundant, is a possible explanation for a smaller uptake of earthworms. For the canton of Ticino, this would most likely be chestnuts, which are very common in that region.

The mean worm burden of *Metastrongylus* spp. in this study was 196.5 nematodes per lung and, like for the prevalence, worm burdens were larger in northern wild boars (218.0, with up to 2895 specimens in a single animal) compared to southern animals (157.4, maximum 597 specimens/animal), although this difference was not significant. Overall, these numbers were significantly higher compared to the ones obtained from wild boars of other European countries, varying from 6.3 to 96.2%, reviewed in Table 5 (de-la-Muela et al., 2001; Fernandez-de--Mera et al., 2003; García-González et al., 2013; Jä;rvis et al., 2007; Nosal et al., 2010; Poglayen et al., 2016). In a study from Spain, lungworm burden of imported captive bred wild boars were much higher than in the autochthonous, free-ranging population, with a mean burden of 633 nematodes per lung (Fernandez-de-Mera et al., 2003). This suggests that with higher animal density, which is mostly inevitable in captivity, the mean lungworm burden of wild boars is higher. Furthermore, areas with regular food supply – a situation typical for animal keeping and game reserves – increase the risk of *Metastrongylus* spp. superinfections, premising the presence of intermediate hosts, i.e. earthworms (Humbert and Henry, 1989). This may seem contradictive, since the wild boars of our study were not captive-bred, and man-made feeding places to generate a higher wild boar density for fruitful hunting are not a common practice in Switzerland (Meier and Ryser-Degiorgis, 2018). Therefore, other factors that were not investigated in this study may be involved causing high lungworm burdens, e.g., regional high occurrence of infected earthworms and rooting behaviour.

No significant difference regarding prevalence (79.4 vs. 76.0%) and mean worm burden (221.9 vs. 177.6%) was observed between male and female wild boars, respectively, indicating the absence of relevant behavioural or immunological divergences that could induce differences regarding lungworm infections. This was also observed in other studies (García-González et al., 2013, Poglayen et al., 2016).

Although not significant, we observed an increase in prevalence from juvenile to subadult animals by trend (from 75 to 86%), indicating an increased uptake of infected earthworms or accumulation of *Metastrongylus* spp. stages in subadult animals. Interestingly, prevalence was lower in adult animals (50%). This was linked with decreasing worm burdens from juvenile (mean: 218.6) to subadult (187.5) and adult (101.0) animals. Similar findings were previously observed in wild boars from Italy (Poglayen et al., 2016), suggesting the development of an acquired immune response with increasing age leading to lower worm burdens (Humbert and Henry, 1989). However, also the opposite, i.e. increasing worm burdens in older animals, have been described (Nosal et al., 2010), indicating that infection intensity is apparently depending on multiple factors. As younger, in particular male, boars tend to leave their pack at a certain age, these animals clearly play an important role in maintenance and spread of *Metastrongylus* spp. infections in the Swiss suid population.

Among *Metastrongylus* spp., the species with the highest prevalence (67.9%) was *M. pudendotectus*, which is equal to the situation in other European countries, where its prevalence was even higher, reviewed in Table 5 (Epe et al., 1997; Jä;rvis et al., 2007; Nosal et al., 2010). *M. pudendotectus* was not only the species with the highest prevalence, but also by far the species with the most individuals, representing 57.1%

Table 5
Mean *Metastrongylus* spp. overall prevalence, worm burden and species prevalence in wild boars from different European countries (n.i.: not identified, no differentiation to species level; n.s.: not specified, presence of a given species is described but not quantified).

Country	Positive animals (N)	Overall prevalence (%)	Mean worm burden	*M. apri* (%)	*M. asymmetricus* (%)	*M. confusus* (%)	*M. pudendotectus* (%)	*M. salmi* (%)	Reference
Germany	45	100.0	65.8	91.1	0	24.5	93.3	80.0	Epe et al. (1997)
Spain	40	85.0	72.2	n.i.	n.i.	n.i.	n.i.	n.i.	De la Muela et al. (2001)
Spain	7	46.7	6.29	n.i.	n.i.	n.i.	n.i.	n.i.	Fernandez-de-Mera et al. (2003)
Estonia	82	82.0	96.2	41.0	0	0	78.0	77.0	Järvis et al. (2007)
Poland	20	80.0	84.8	64.0	40.0	76.0	76.0	72.0	Nosal et al. (2010)
Spain	381	41.1	27.0	n.s.	0	0	n.s.	n.s.	García-Gonzáles et al. (2013)
Switzerland	65	77.4	196.5	44.0	17.9	56.0	67.9	63.1	Present study

of all *Metastrongylus* specimens determined in this study, which makes *M. pudendotectus* the dominant species in Swiss wild boars.

The second most abundant *Metastrongylus* species was *M. salmi,* with a still high prevalence (63.5%), followed by *M. confusus* (56.0%). Finding *M. salmi* to be the second most common species also coincides with studies from Estonia (Jä;rvis et al., 2007), Poland (Nosal et al., 2010) and Italy (Poglayen et al., 2016). For these, *M. pudendotectus, M. salmi* and *M. confusus,* prevalences in the north and in the south of Switzerland were very similar. In contrast, *M. apri* was significantly more common in southern Switzerland (62.5%) than in the northern parts of the country (32.7%). This parasite was detected with prevalences ranging from 23% to 91.1% all over Europe in the last decades (Poglayen et al., 2016). Overall, differences regarding *Metastrongylus* spp. communities in Swiss wild boars as well as in other European wild boar populations illustrate that the composition of *Metastrongylus* spp. infections can be highly variable and that certain species are indeed more established in some areas, whereas they may rarely occur in others. This may depend on numerous exo- and endogenous factors, i.e., environmental and biological factors. We hypothesize that the higher prevalence of *M. apri* south of the Alps could be explained by a better adaptation to warmer climate with a higher number of annual sun hours in that region. Similar climatic preferences can be found in other nematodes, such as for canine hook worms, with *Uncinaria stenocephala* being much more prevalent in warmer regions and *Ancylostoma caninum U. stenocephala* more prevalent in cooler climates (Deplazes et al., 2013). Additionally, interactions between the different *Metastrongylus* species may have an impact on the composition of a lungworm infection in wild boars and pigs, as some authors suggest mutualism between certain parasite species (Ewing et al., 1982). The overall sex ratio in favour of female nematodes (1 : 2.23), consistently in both examined wild boar populations and within all *Metastrongylus* species, with the exception of *M. asymmetricus* in the southern wild boar population (1 : 0.79). This inconsistency indicates that such ratios are not irrevocable but may be influenced by many intrinsic and extrinsic factors (Kloch et al., 2015). In our study only 25 specimens of *M. asymmetricus* were found in the southern wild boar population, therefore a bias due to small sample size cannot be excluded. Female dominated *Metastrongylus* infections were previously identified in numerous other studies in wild boars (García-González et al., 2013; Nosal et al., 2010; Poglayen et al., 2016). Various hypotheses explaining this phenomenon were discussed, one being a longer lifespan of female parasites (Poulin, 1997; Roche and Patrzek, 1966). Alternatively, female biased population were suggested to reduce inbreeding, although further studies are necessary regarding the genetic relationship of the nematodes in a single animal (Kloch et al., 2015). Not least, male nematodes are much smaller and may be therefore overlooked more easily, which can also artificially lead to a female biased population (Pence et al., 1988).

Regarding the effects of *Metastrongylus* sp. infection on the health of wild boars, definitive assertions are not possible, since none of the sampled animals were monitored for clinical signs while they were alive. However, hypotheses about the possible relevance of lungworm infections can be deduced from the gross macroscopic lung examinations. Here, we found that 29.3% of *Metastrongylus* positive animals had dense nodules, especially in lung areas where many *Metastrongylus* specimens were present. Since these affected areas were always rather small and not diffusely distributed, a relevant restriction of the lung function through these lesions is questioned. It is indeed described that lungworm infection can proceed without apparent or only very mild clinical signs in pigs (Deplazes et al., 2013). A certain symbiosis with a relatively high tolerance for the parasite could be hypothesized, as it is known for example with the canid heart and lung nematode *Angiostrongylus vasorum* in foxes (Woolsey et al., 2017; Gillis-Germitsch et al., 2020). However, especially in juvenile animals, having smaller bronchi and bronchioles, conglomerates of *Metastrongylus* spp. often completely filled out the lumen of the airways in one or more areas of the lung. It can be assumed that such an obstruction with nematodes can easily lead to non-ventilated areas in the lung and subsequently to respiratory symptoms such as dyspnoea (Deplazes et al., 2013). Additionally, in 14 animals the bronchiolar walls were thickened in areas with high *Metastrongylus* sp. abundance, which will most likely aggravate airflow issues. Three of the sampled lungs showed signs of a secondary bacterial infection with purulent matter found in the bronchioles: clinically, this type of infection is associated with a reduced general condition and fever, among other symptoms (Zachary and McGavin, 2012). In summary, although not observable, apparent and/or non-apparent clinical signs determining the health may have been occurring in some of the infected wild boars. Little is known regarding immune mechanisms against metastrongylids in wild boars; experimental infections may provide additional knowledge and therefore help to understand e.g., the lack of significant differences between hosts of different age groups observed in our study.

A variety of endoparasites was detected in the faecal samples, with only two animals without any parasitic stages detected. High parasite variety and burden in wild animals is common since they rely on food potentially contaminated with parasites and are normally not treated with antiparasitics. Conventional pig meat industry in Switzerland mainly relies on animals kept indoors, with outdoor areas generally based on concrete floors. Currently, in the frame of organic farming and for animal welfare reasons, an increasing number of free-ranging farm pigs can be observed. Their typical fodder also consists of specific rations for each age group, so these animals are not depending on rooting for food (Agroscope, 2004) and therefore an oral uptake of e.g., earthworms is less likely. However, due to their natural rooting behaviour they are nevertheless at higher risk of infection for parasitic infections, despite routine anthelmintic treatments.

All sampled animals with positive faecal samples for *Metastrongylus* spp. also had nematodes in the lung, so a prolific infection with *Metastrongylus* spp. was confirmed. There was no quantitative correlation between the *Metastrongylus* spp. worm burdens and the amount of *Metastrongylus* spp. eggs shed in the faeces. Such non-correlated endoparasite burden and egg shedding is common in multiple other host and parasite species (Deplazes et al., 2013; Ngongeh, 2017).

By far the most abundant parasites detected in faeces, besides *Metastrongylus* spp. (72.7%), were *Eimeria* sp./*Isospora suis* (74.5%), which was also frequently found in wild boars from other European countries (Epe et al., 1997; Oja et al., 2017). In pigs, *Eimeria* spp. infections are mostly not inducing clinical signs, and *Isospora suis* infections only cause symptoms in young piglets, while older animals develop an immunity with the first infection (Deplazes et al., 2013). None of the positive wild boars showed diarrhoea, which is the leading symptom of coccidiosis. Since wild boars belong to the same species as domestic pigs and none of the sampled animals were shoats (wild boar piglets), it can be assumed that these *Eimeria* sp./*Isospora suis* infections were clinically inapparent and did not affect the health of the animals but their role as reservoirs for these protozoic parasites may be relevant in the broader context of suid health, since domestic pigs can develop severe symptoms which consequent economic losses (Deplazes et al., 2013).

Other parasite species found in Swiss wild boars in this study were *A. suum*, *B. coli*, *Capillaria* sp., *Globocephalus* sp., *Hyostrongylus rubidus*/*Oesophagostomum* sp., *S. suis* and *T. suis*, which are common in wild boars in Europe (Barutzki et al., 1990; Epe et al., 1997; Fernandez-de-Mera et al., 2003; Foata et al., 2006; Jä;rvis et al., 2007; Navarro-Gonzalez et al., 2013; Oja et al., 2017). Significant differences between the two Swiss wild boar populations were observed with *Globocephalus* sp. and *S. suis* infections. *Globocephalus* sp. eggs were only detected in faecal samples from the southern populations (26.3%), indicating an endemic local presence of this nematode. However, *Globocephalus* sp. does occur north of the Alps and in eastern Europe as well (Barutzki, 1990; Panayotova-Pencheva and Dakova, 2018), but was not identified in suckling and weaning piglets and fatteners in a recent study on Swiss pigs (Schubnell et al., 2016) nor in wild boar kept in captivity in 10 zoos and wild parks north of the Alps (Basso, 2015). In contrast, we observed a lower prevalence of *S. suis* in the southern population (10.5%) compared to the northern population (36.1%) that could be related to the higher amount of annual sun hours in this region (Federal Office of Meteorology and Climatology, 2019) and the sensitivity of *S. suis* third stage larvae for dryness (Deplazes et al., 2013), or other unknown biogeographic factors. This parasite was also absent in Swiss pigs and wild boar kept in captivity (Schubnell et al., 2016). Interestingly, in the last PathoPig report, a Swiss surveillance program that included animals of 971 pig farms in 2019 (Federal Food Safety and Veterinary Office, 2020), in a single animal with gastro-intestinal disorders a high number of eggs and nematode sections were identified in the histological intestinal sections at necropsy, representing presumably the first detection of this parasite in a Swiss pig.

Furthermore, *T. suis* (20.0%) and *Hyostrongylus rubidus/Oesophagostomum* sp. (18.2%) were frequently detected, both parasites that are also occasionally present, i.e. in 2.7% of the pig farms (Schubnell et al., 2016) and in wild boars kept in captivity (Basso, 2015). These parasites have a direct life cycle and may induce gastritis (*H. rubidus*) or damage the small (*Globocephalus* sp.) or the large (*T. suis, Oesophagostomum* sp.) intestinal wall. *Ascaris suum* (detected in two animals north of the Alps) also has a direct life cycle and has a zoonotic relevance. This parasite, causing the so-called "milk-spots" in the liver during migration, was rarely identified in 0.8% of pig farms and in two out of 10 parks keeping wild boars in captivity (Schubnell et al., 2016).

Capillaria sp. has previously described in wild boars from Spain (de-la-Muela et al., 2001; Fernandez-de-Mera et al., 2003), where it has been determined as *Capillaria garfiai*, and in Germany in wild boars kept in captivity (Barutzki et al., 1990, 1991), but oral uptake of other *Capillaria* sp. eggs and passage through the digestive system without an actual infection cannot be excluded (Deplazes et al., 2013).

Eventually, despite faecal flotation not being the most suitable method for their detection, the protozoans *Giardia* sp. and *B. coli* were occasionally detected; their clinical relevance is negligible.

6. Conclusion

In this study we identified high prevalences of *Metastrongylus* spp. in two separated Swiss wild boar populations living north and south of the Alps. Overall, all five globally occurring *Metastrongylus* species were present, leading to high worm burdens dominated by *M. pudendotectus*. Male and female animals were equally infected and there was no significant difference regarding age, although the development of immunity against *Metastrongylus* spp with age can be assumed. Macroscopically visible pathological changes in the lungs were present in particular in the caudal lobi of the lungs. However, as the observation of clinical signs in wild boars is challenging, their clinical relevance can be questioned, while assuming a certain tolerance or even symbiosis.

The comparison of lung necropsy results with egg shedding allowed us to conclude that the number of *Metastrongylus* sp. eggs does not correlate with the worm burden. The high prevalences and parasite variety detected in faeces, combined with successful reproduction and migration tendencies of wild boars help to maintain a broad parasite spectrum and geographic spread of *Metastrongylus* spp. and other infections. Although geographically separated, both Swiss wild boar populations share similar parasite spectra, while also showing some regional idiosyncrasies. Such accurate knowledge concerning the distribution of certain pathogens in wildlife also contributes to better understand risk factors for the domestic pig population.

Declaration of competing interest

The authors declare no competing interests.

Ethics

Ethical standards were fulfilled corresponding to national laws.

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